RESEARCH

Time trends in drug resistant HIV-1 infections in the United Kingdom up to 2009: multicentre observational study

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UK Collaborative Group on HIV Drug Resistance

Abstract

Objective To evaluate whether the prevalence of HIV-1 transmitted drug resistance has continued to decline in infections probably acquired within the United Kingdom.

Design Multicentre observational study.

Setting All UK public laboratories conducting tests for genotypic HIV resistance as a part of routine care.

Participants 14 584 patients infected with HIV-1 subtype B virus, who were first tested for resistance before receiving antiretroviral therapy between January 2002 and December 2009.

Main outcome measure Prevalence of transmitted drug resistance, defined as one or more resistance mutations from the surveillance list recommended by the World Health Organization.

Results 1654 (11.3%, 95% confidence interval 10.8% to 11.9%) patients had one or more mutations associated with transmitted HIV-1 drug resistance; prevalence was found to decline from 15.5% in 2002 to 9.6% in 2007, followed by a slight increase to 10.9% in 2009 (P=0.21). This later rise was mainly a result of increases in resistance to nucleos(t)ide reverse transcriptase inhibitors (from 5.4% in 2007 to 6.6% in 2009, P=0.24) and protease inhibitors (1.5% to 2.1%, P=0.12). Thymidine analogue mutations, including T215 revertants, remained the most frequent mutations associated with nucleos(t)ide reverse transcriptase inhibitors, despite a considerable fall in stavudine and zidovudine use between 2002 and 2009 (from 29.4% of drug regimens in 2002 to 0.8% in 2009, from 47.9% to 8.8%, respectively).

Conclusions The previously observed decline in the prevalence of transmitted drug resistance in HIV-1 infections probably acquired in the UK seems to have stabilised. The continued high prevalence of thymidine analogue mutations suggests that the source of this resistance may be increasingly from patients who have not undergone antiretroviral therapy and who harbour resistant viruses. Testing of all newly diagnosed HIV-1 positive people should be continued.

Introduction

Combination antiretroviral therapy continues to be highly effective in treating HIV-1, and the introduction of new drugs and antiretroviral drug classes has notably improved patient prognosis. Nevertheless, resistance to antiretroviral drugs can develop in people on therapy and is associated with treatment failure.[1] Against this backdrop, the rate of new HIV-1 infections within the United Kingdom continues to rise.[2] In such new infections, transmitted drug resistance is sometimes assumed to reflect only direct infection from patients already receiving antiretroviral therapy. Concerns about an adverse effect of transmitted HIV-1 drug resistance on the success of antiretroviral therapy have led to national and international guidelines recommending that all newly diagnosed patients have resistance tests conducted to aid selection of first line regimens.[3 4]

A previous study showed a sharp decline in the prevalence of transmitted HIV-1 drug resistance in the UK between 2002 and 2005,[5] which was mainly attributed to changes in testing guidelines and the wider use of regimens that suppress viral concentrations to below infectious levels. Since then, more potent and better tolerated antiretroviral drugs have been introduced, and the proportion of patients achieving viral suppression has continued to increase.[6] This suggests that transmitted HIV-1 drug resistance may have declined even further and could eventually fall below levels in which universal testing before antiretroviral therapy is cost effective,[7] an important issue at a time when the cost of HIV-1 management is being scrutinised.[8] This paper examines recent time trends in transmitted drug resistance in HIV-1 infections probably acquired in the UK.

Methods

Resistance data

The UK HIV Drug Resistance Database, described in detail elsewhere,[9] was established in 2001 and collects the majority of genotypic resistance tests done within the UK as part of routine clinical care. The resistance tests analysed in this study used bulk sequencing of the *pol* gene, encoding at least codons 4-99 of the protease gene and 34-234 of the reverse transcriptase gene, using a variety of inhouse and commercial testing systems. Subtype was assigned centrally using the Rega algorithm.[10]

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Clinical data

We acquired demographic and clinical information by linkage (using pseudonymised identifiers) to the UK Collaborative HIV Cohort Study (UK CHIC),[9] which includes patients from 13 of the largest clinics within the UK, and to the HIV and AIDS Patient and the Survey of Prevalent HIV Infections Diagnosed databases, which are coordinated by the Health Protection Agency. When possible, we linked resistance tests done after 2007 to samples on which a recent infection testing algorithm had been conducted as part of a national health surveillance programme[11]; these tests use antibody avidity assays to classify infections as either recent (probably occurring in the previous five months) or non-recent;[12] and clinical, laboratory, or historical information to reduce the false recent rate.

Tests included in analysis and definition of drug resistance

The UK has several parallel and largely non-overlapping HIV-1 epidemics with different levels and patterns of resistance to antiretroviral therapy.[13 14] To simplify the understanding of temporal trends, we limited this analysis to subtype B viral infections, so it is not intended to generate nationally representative results. This epidemic was seeded by around six introductions to the UK in the early to mid-1980s[15] and is largely confined to men infected through homosexual exposure, of whom 83% are estimated to have acquired infection within the UK.[16]

We identified the first resistance test for all patients older than 16 years who had not yet received antiretroviral therapy at the time of sampling, up to the end of 2009. Patients with an undetectable viral load (<50 copies/mL) were excluded; such levels may indicate unrecorded treatment use. Since guidelines in 2001 first recommended that resistance tests be performed for all patients who had not received antiretroviral therapy,[3] we excluded tests conducted before 2002.

Transmitted HIV-1 drug resistance was defined as one or more mutations from the surveillance list recommended by the World Health Organization.[17] We used the Stanford HIVdb algorithm 6.0.11 (29 Mar 2011) to examine susceptibility to antiretroviral drugs, and reported low level resistance or greater. Intermediate or high level resistance was considered to reflect a substantial loss in susceptibility. We assessed temporal trends in terms of the date of the resistance test sample rather than the date of the patient's infection, which is generally not known.

Statistical methods

Confidence intervals for proportions were calculated using a 95% Wilson confidence interval for binomially distributed data. We analysed the patterns of trends over continuous time using both linear and piecewise linear logistic regression with a flexible choice of a single inflexion point calculated using least squares optimisation; we selected the model with the best fit according to Akaike's information criterion. The trends for codons were reported if a mutation had an overall prevalence of more than 0.3% as well as other mutations for nucleos(t)ide reverse transcriptase with a strong effect on phenotype (K65R, K70E, L74I/V, Y115F). We examined differences between the prevalence of resistance in recent and non-recent infections tests using the χ^2 test. All statistical analyses were conducted in Stata/IC 11.2 software.

Results

Population characteristics

We analysed 14 583 patients who were antiretroviral therapy naive, infected with a subtype B virus, and whose first drug resistance test was conducted between January 2002 and December 2009. Of these patients, 10 173 (70%) were white, 995 (7%) were black, 711 (5%) had a known other ethnicity, and 2704 (19%) had an unknown ethnicity. The median age at diagnosis was 36 years (interquartile range 30 to 42). Of the patients analysed, 10 288 (71%) were men who had sex with men, 1275 (9%) had a heterosexual exposure source, 313 (2%) had a known other exposure source, and 2707 (19%) had an unknown exposure source. The median number of days between HIV-1 diagnosis and resistance sample was 22 (interquartile range five to 358). The median CD4 count at the time of testing, available from 11 219 (76.9%) patients, was 408×10^6 cells/L (interquartile range 271×10^6 to 560×10^6).

The number of resistance tests conducted per year increased over time, reflecting a rise in the number of new diagnoses among men who have sex with men (fig 1⇓). The decline between 2008 and 2009 is mainly due to an increase in the proportion of tests where the patient's status regarding antiretroviral therapy exposure was uncertain (15.6% in 2008 to 32.0% in 2009), probably as a result of a reporting lag with demographic and clinical datasets.

Trends in transmitted drug resistance

Samples from 1654 (11.3%, 95% confidence interval 10.8% to 11.9%) patients had one or more mutations associated with transmitted HIV-1 drug resistance. Of these samples, 1009 (6.9%, 6.5% to 7.3%), 604 (4.1%, 3.8% to 4.5%), and 319 (2.2%, 2.0% to 2.4%) had one or more mutations associated with nucleos(t)ide reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and protease inhibitors, respectively. Of these samples, 1426 (9.8%, 9.3% to 10.3%) had single class resistance, 175 (1.2%, 1.0% to 1.4%) had dual class resistance, and 52 (0.4%, 0.3% to 0.5%) had triple class resistance; dual and triple class resistance have remained at a similar prevalence since 2005.

The previously reported[5] decline in the prevalence of transmitted HIV-1 drug resistance for any class of antiretroviral drug was observed to continue from 15.5% in 2002 until around January 2007 (95% confidence interval January 2006 to February 2008; odds ratio 0.88 per year (95% confidence interval 0.84 to 0.92); fig 1). However, between 2007 and 2009, we saw a non-significant increase from 9.6% to 10.9% (odds ratio 1.06, 0.97 to 1.17; P=0.21). We saw non-significant increases in resistance to nucleos(t)ide reverse transcriptase inhibitors (from 5.4% in 2007 to 6.6% in 2009; P=0.24) and protease inhibitors (1.5% to 2.1%; P=0.12). Inflection points were also identified, which gave a significantly better fit for mutations associated with nucleos(t)ide reverse transcriptase inhibitors (February 2007 (January 2006 to March 2008); second piece odds ratio 1.08 (0.95 to 1.22)) and protease inhibitors (June 2008 (August 2007 to June 2009); 1.69 (0.86 to 3.31)). The prevalence of mutations associated with non-nucleoside reverse transcriptase inhibitors remained stable at around 3.6% with no evidence of non-linearity (odds ratio 0.96 (0.92 to 1.00)).

The table⇓ displays the resistance trends over time for key individual codons with prevalence greater than 0.3%. The trends in HIV-1 drug resistance associated with nucleos(t)ide reverse transcriptase inhibitors largely reflect the most common mutations within this drug class, namely, the T215 revertant[18]

mutations (I/S/C/D/V/E), K219Q/E/N/R, and M41L. These mutations are either reversions or a subset of the thymidine analogue mutations that develop under regimens containing either stavudine or zidovudine. The finding of a levelling off in the prevalence of thymidine analogue mutations is paradoxical, in the light of the dramatic fall in the use of stavudine and zidovudine. For example, in the UK CHIC study, the proportion of treatment regimens that included stavudine dropped from 29.4% to 0.8% between 2002 and 2009, while the proportion that included zidovudine dropped from 47.9% to 8.8% over the same period. Stavudine and zidovudine have largely been replaced by tenofovir and abacavir,[3] but signature mutations for these drugs (such as K65R) are still rare in patients with HIV-1. L90M mutations have increased in prevalence since 2007, despite the near cessation in the use of saquinavir, nelfinavir, and indinavir, which are first generation protease inhibitors that select for this mutation (their use in the UK as part of a drug regimen fell from 20.6% in 2002 to 4.6% in 2009), although L90M has broad cross resistance effects to the protease inhibitor class.[19]

Drug susceptibility

Figure 2⇓ shows the predicted susceptibility of HIV-1 from samples collected in 2009 to currently recommended first line antiretroviral drugs[3] and second generation antiretroviral drugs darunavir and etravirine. The association between genotypic mutations and the phenotypic susceptibility to antiretroviral drugs is complex, so although T215 revertants are considered by the Stanford HIVdb algorithm to result in only low level resistance to nucleos(t)ide reverse transcriptase inhibitors, they do confer a low genetic barrier to the development of high level resistance to this drug class.

The prevalence of intermediate or high level resistance to nucleos(t)ide reverse transcriptase inhibitors other than zidovudine was less than 0.9%, reflecting the low frequency of multiple mutations in thymidine analogues. There was a comparatively high level of reduced susceptibility to recommended first line regimens of the non-nucleoside reverse transcriptase inhibitors efavirenz and nevirapine (3.7%). However, etravirine, a non-nucleoside reverse transcriptase inhibitor, displayed very low levels of resistance (0.4%), reflecting the lack of predicted effect of K103N. The potency of modern drugs from the protease inhibitor class was high (only 25 (1.1%) patients had substantially reduced susceptibility to any protease inhibitor), owing to the rarity of multiple protease mutations. These findings indicate that potent first line regimens can still be constructed from the original three drug classes for almost all patients.

Prevalence of resistance in recently infected patients

Between 2007 and 2009, 742 samples were linked to a recent infection test result, of which 171 (23.0%) were classified as recent. The overall prevalence of resistance did not differ significantly (P=0.66) between recent samples (20, 11.7%) or non-recent samples (60/571, 10.5%). Furthermore, we detected no significant differences in the prevalence of resistance by individual drug class (results not shown).

Discussion

Interpretation

The previously observed decline in the prevalence of transmitted drug resistance in subtype B viruses seems to have been reversed for mutations associated with nucleos(t)ide reverse transcriptase inhibitors and protease inhibitors, despite an increase in the proportion of patients on antiretroviral therapy who are virologically suppressed (from 62% in 2000 to 84% in 2007[6]). The most frequent mutations were T215 revertants, which may be transmitted as such or evolve from a virus harbouring a T215F or T215Y mutation.[19] These and other mutations are associated with a significantly higher risk of virological failure than wild type genotypes.[1 17] The prevalence of transmitted thymidine analogue mutations remained moderately high, despite a marked shift away from the prescribing in the UK of drugs that select for these mutations.

One plausible explanation for this paradox is the onward transmission of resistant viruses from people who have not received antiretroviral therapy before and who were themselves infected with a resistant virus. This hypothesis is supported by a previous phylogenetic analysis of UK subtype B sequences, which described five transmission clusters comprised exclusively of patients with resistance mutations who are antiretroviral therapy naive.[20] It was postulated that an increasingly greater proportion of transmitted HIV-1 drug resistance could originate from antiretroviral therapy naive lineages, and that there could ultimately be a limit in the decline of transmitted HIV-1 drug resistance.

The first factor contributing to this possible limit is that people with undiagnosed HIV could disproportionally[21] spread the epidemic, since they are more infectious in the period immediately after infection.[22] Some studies[23 24] (but not all[25]) have also shown that undiagnosed patients with HIV have more sexual partners than diagnosed patients. Furthermore, evidence is emerging that transmitted resistant viruses are more persistent than originally thought. The fitness cost (relative to wild type virus) of certain mutations, such as the T215 revertants and K103N, has been shown to be marginal in laboratory studies.[26 27] Jain and colleagues provided clinical confirmation of this finding in a series of patients infected with resistant virus and who had two or more resistance tests before they started antiretroviral therapy.[28] With the exception of the M184V mutation, which is highly replicatively deficient, all groups of transmitted mutations persisted beyond at least three years in the majority of patients.

A second possible explanation for the continued prevalence of thymidine analogue mutations is that the use of tenofovir and abacavir are maintaining the prevalence of such mutations in patients who have received antiretroviral therapy despite the decline in the use of zidovudine and stavudine. Further phylogenetic research could shed light on the transmission dynamics of these mutations.

Comparison with other studies

Two recent studies have reported on time trends in transmitted HIV drug resistance. In a study conducted in 20 European countries between 2002 and 2006,[29] Vercauteren and colleagues found a small, linear decline in levels of nucleos(t)ide reverse transcriptase inhibitor and protease inhibitor resistance; non-nucleoside reverse transcriptase inhibitor resistance was observed to increase followed by a decrease between 2004 and 2006. Bartmeyer and colleagues[30] performed a similar analysis of a German seroconverter cohort between 1996 and 2007. In more recent years, resistance to nucleos(t)ide reverse transcriptase inhibitors seemed to be stable and resistance to non-nucleoside reverse transcriptase inhibitors seemed to increase, although clear patterns are difficult to discern due to the relatively small sample size.

Study limitations

Our study has several limitations. Firstly, the analysis is based on resistance found at date of sample rather than date of infection. Since viral quasi-species harbouring resistance mutations may revert to, or be overgrown by, virus without the mutations,[19][29] the true level of transmitted HIV drug resistance may have been under-estimated in this analysis. Also, the degree of this bias will be affected by the average time between HIV-1 infection and diagnosis, which may have changed over time. Furthermore, the diagnosis delay could mask the underlying trend in the prevalence of transmitted resistance by date of infection. However, we found no difference in the prevalence of resistance between recent and non-recent infections, in the subset of patients in which this analysis was possible.

Secondly, the genotypic data analysed were generated by population sequencing with a limit of sensitivity of approximately 15 to 25%.[31][32] Our estimates of the prevalence of transmitted HIV drug resistance may therefore be biased downwards. This will have also biased the type of mutations observed, with persistent mutations appearing to be more prevalent than those which rapidly become undetectable such as K65R or M184V. We do not consider this limitation to be a major concern, because the main objective of our study was to examine changes in the prevalence of transmitted HIV drug resistance over time.

Another limitation was that our method of classifying treatment status could have resulted in misclassification bias if some patients who had received antiretroviral therapy were included in the analysis. Previous research by the UK HIV Drug Resistance Database has suggested that this effect could distort trends if there is misclassification in 4% or more of the samples analysed.[33] Finally, as the prevalence of transmitted HIV drug resistance in subtype B viruses, the focus of this analysis, is known to be higher than that observed in other subtypes,[34] our findings are not generalisable to the UK epidemic as a whole, although coverage of patients infected with subtype B virus is high.

Conclusions and policy implications

Finally, we consider the clinical implications of our main conclusion that resistant lineages may have become fixed in the circulating viral pool. This concept, if confirmed to be correct, will apply universally, particularly in countries where first generation nucleos(t)ide reverse transcriptase inhibitors continue to be used, and underscores the importance of sentinel surveillance. In terms of the UK (and probably other well resourced countries), the detectable mutations that tend to be transmitted should have little effect on nucleos(t)ide reverse transcriptase inhibitors currently used in first line regimens, that is, abacavir, tenofovir, lamivudine, and emtricitabine. Non-nucleoside reverse transcriptase inhibitors used in first line regimens are of greater concern, with approximately 4% of patients being infected with viruses with reduced susceptibility to efavirenz and nevirapine. Previous models[7] have suggested that baseline resistance testing remains cost effective at the levels observed in this study. Therefore, our findings argue that testing at HIV diagnosis and continued monitoring should remain the standard of care.

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What is already known on this topic

Transmitted HIV drug resistance can affect therapy success

Some resistance mutations could persist more than others in the absence of selective drug pressure

A 2007 paper has shown a reduction in transmitted drug resistance in the UK since 2005

What this study adds

Transmitted drug resistance is no longer declining in UK, and evidence suggests a sustained epidemic that is resistant to nucleos(t)ide reverse transcriptase inhibitors, irrespective of previous antiretroviral therapy use

Susceptibility to antiretroviral therapy remains relatively high, and potent first line regimens can still be constructed from the original three drug classes for almost all patients

programme; no financial relationships with any organisations that might have an interest in the submitted work in the previous 3 years; and no other relationships or activities that could appear to have influenced the submitted work.

Ethical approval: This study was approved by the UK multicentre research ethics committee and relevant local research ethic committees.

Data sharing: No additional data available.

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Table

Table 1| Time trends of selected mutations with prevalence greater than 0.3%. Data are no (%) of samples with mutation unless stated otherwise

Mutation	Year (no of samples)							
	2002 (n=547)	2003 (n=766)	2004 (n=1364)	2005 (n=2054)	2006 (n=2455)	2007 (n=2525)	2008 (n=2651)	2009 (n=2221)
Protease inhibitors								
M46I/L	12 (2.19)	9 (1.17)	13 (0.95)	17 (0.83)	14 (0.57)	14 (0.55)	15 (0.57)	13 (0.59)
V82A/T/F/S/C/M	9 (1.65)	10 (1.31)	19 (1.39)	12 (0.58)	7 (0.29)	8 (0.32)	3 (0.11)	7 (0.32)
L90M	8 (1.46)	9 (1.17)	20 (1.47)	24 (1.17)	18 (0.73)	12 (0.48)	16 (0.60)	21 (0.95)
Non-nucleoside reverse transcriptase inhibitors								
K103N/S	21 (3.84)	28 (3.66)	40 (2.93)	57 (2.78)	67 (2.73)	76 (3.01)	89 (3.36)	62 (2.79)
Y181C/I/V	5 (0.91)	9 (1.17)	10 (0.73)	17 (0.83)	11 (0.45)	13 (0.51)	12 (0.45)	9 (0.41)
G190A/S/E	7 (1.28)	7 (0.91)	10 (0.73)	14 (0.68)	9 (0.37)	8 (0.32)	8 (0.3)	3 (0.14)
Nucleos(t)ide reverse transcriptase inhibitors								
M41L	27 (4.94)	17 (2.22)	46 (3.37)	49 (2.39)	49 (2.00)	41 (1.62)	51 (1.92)	33 (1.49)
K65R	1 (0.18)	3 (0.39)	3 (0.22)	1 (0.05)	1 (0.04)	3 (0.12)	3 (0.11)	0
D67N/G/E	12 (2.19)	9 (1.17)	20 (1.47)	21 (1.02)	21 (0.86)	13 (0.51)	13 (0.49)	9 (0.41)
K70E*	6 (1.10)	9 (1.17)	4 (0.29)	5 (0.24)	6 (0.24)	2 (0.08)	4 (0.15)	3 (0.14)
L74I/V*	5 (0.91)	3 (0.39)	3 (0.22)	5 (0.24)	4 (0.16)	3 (0.12)	2 (0.08)	1 (0.05)
Y115F*	0	2 (0.26)	1 (0.07)	1 (0.05)	1 (0.04)	0	1 (0.04)	2 (0.09)
M184I/V	11 (2.01)	13 (1.70)	15 (1.1)	15 (0.73)	13 (0.53)	14 (0.55)	9 (0.34)	7 (0.32)
L210W	12 (2.19)	7 (0.91)	15 (1.1)	13 (0.63)	12 (0.49)	9 (0.36)	12 (0.45)	8 (0.36)
T215Y/F	12 (2.19)	9 (1.17)	9 (0.66)	17 (0.83)	7 (0.29)	3 (0.12)	2 (0.08)	2 (0.09)
T215I/S/C/D/V/E	35 (6.40)	35 (4.57)	59 (4.33)	82 (3.99)	86 (3.50)	82 (3.25)	102 (3.85)	87 (3.92)
K219Q/E/N/R	10 (1.83)	19 (2.48)	31 (2.27)	34 (1.66)	34 (1.38)	33 (1.31)	41 (1.55)	39 (1.76)

*Included because these are major mutations to recommended first line antiretroviral drugs.
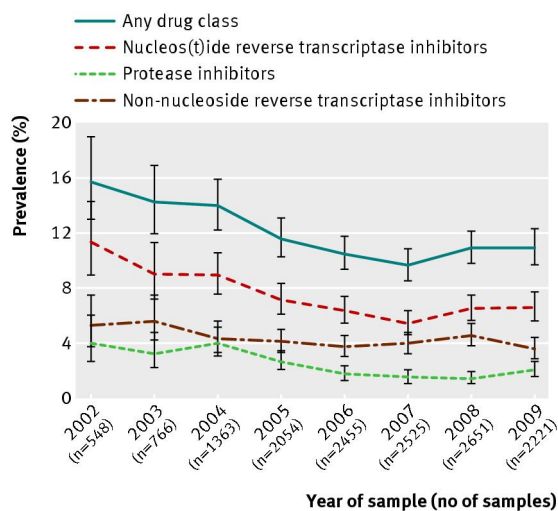
Figures

Fig 1 Prevalence of transmitted drug resistance over time, by antiretroviral drug class. Bar=95% confidence interval
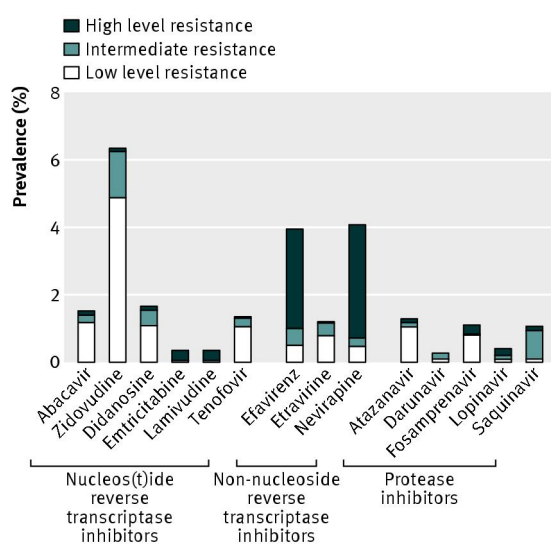

Fig 2 Predicted susceptibility to antiretroviral drugs